Standardization and clinical implementation of liquid biopsy assays - IMI's CANCER-ID

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INTRODUCTION

- CANCER-ID (www.cancer-id.eu) is a five-year (2015-2019) international public-private partnership project supported by Europe's Innovative Medicines Initiative (IMI). The consortium of currently 40 partners from 14 countries (**Fig. 1**) aims at the establishment of harmonized best practice protocols for patient sample collection, pre-analytical sample handling, sample and bioinformative analyses, and actionable information guiding patient selection for personalized treatment.
- CANCER-ID tests and supports the development of standards for liquid biopsy as well as clinical implementation of liquid biopsy-based protocols in the clinical setting. This includes interaction with regulatory bodies, such as EMA's (European Medicines Agency) Innovation Task Force (ITF) and CDER/FDA's (U.S. Center for Drug Evaluation and Research/Food and Drug Administration) Critical Path Innovation Meeting (CPIM), to support future approval of liquid biopsies in multi-centered worldwide clinical studies.
- At the core of CANCER-ID's activities in the liquid biopsy field is the evaluation of technologies for circulating tumor cell (CTC), circulating tumor DNA (ctDNA), microRNA (miRNA) and exosome enrichment, isolation and analysis.
- Liquid biopsy protocols are being implemented in an observational study evaluating the utility of analyzing PD-L1 (programmed death-ligand 1) expression on CTCs in non-small cell lung cancer (NSCLC) and metastatic breast cancer. To this end, the potential predictive value of monitoring treatment response towards immune checkpoint inhibition (ICI) is assessed in advanced NSCLC patients at the University Medical Center Groningen (UMCG) as well as in two ICI-chemotherapy combination studies in triple-negative breast cancer and luminal B breast cancer, respectively, run by the University of Oslo (ALICE, ClinicalTrials.gov ID: NCT03164993 and ICON, ClinicalTrials.gov ID: NCT03409198).
- The aim is to assess whether the allelic frequency of mutations as a potential measure for tumor mutational burden (TMB) or the number of PD-L1-positive/overall CTCs at different time points is indicative of treatment success.
- As a follow-up activity of the CANCER-ID program, the European Liquid Biopsy Society (ELBS) is currently being established. The ELBS will be open to all interested liquid biopsy stakeholders worldwide as a platform for scientific exchange.

The CANCER-ID consortium

- The CANCER-ID consortium is funded by IMI (**Fig. 1**). This public-private partnership between the EU commission and the European Federation of Pharmaceutical Industries and Associations (EFPIA) provides a legal framework for addressing unmet challenges in the healthcare sector.
- In 2015, academic and clinical research groups, public research organizations, small and medium-sized enterprises (SME), and pharmaceutical and diagnostic corporations joined forces to evaluate technologies and establish analytical standards in the liquid biopsy field.
- The academic leaders of CANCER-ID, Professor Klaus Pantel (UKE, Germany), who has published >300 reports and high-impact review articles on disseminating tumor cells, and Professor Leon Terstappen (Universiteit Twente, The Netherlands), developer of the FDA-approved benchmark CELLSEARCH® CTC detection system, are pioneers in the field of blood-based cancer biomarkers.

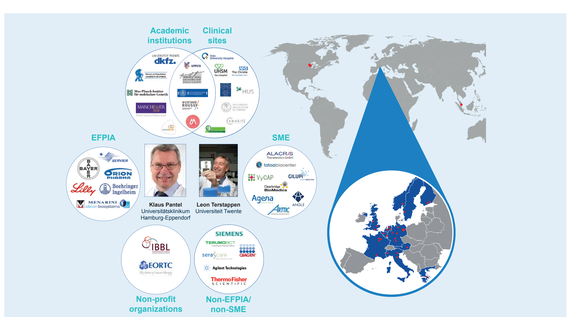

Figure 1. The CANCER-ID consortium, funded by IMI.

Standardization of liquid biopsy technologies

- The use of diverse input sample types (e.g. different blood fixatives, extraction protocols or analysis technologies) and substantially different user-developed protocols for blood-based analytes like CTCs, ctDNA or miRNA hampers the comparability of results (**Fig. 2**).
- Hence, there is a need to standardize liquid biopsy technologies. The multicenter ring trials for the evaluation of CTC, ctDNA and miRNA technologies include the analysis of standard materials (e.g. well-defined NSCLC spike-in controls, ctDNA reference material) by multiple CANCER-ID partners following a consensus protocol or workflow to ensure the comparability of results [1, 2].

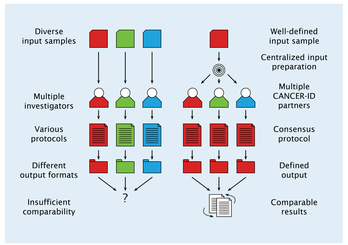

Figure 2. Comparison of workflows using either diverse input samples and protocols or well-defined input samples and consensus protocols.

Efforts to improve the CTC yield in NSCLC patients

- To improve the CTC yield in NSCLC patients, new workflows were established using Diagnostic Leukapheresis (DLA) [3, 4] (**Fig. 3**). CTC detection frequency was significantly increased (p=0.03, Friedman's 2-way ANOVA by rank) when measured in a DLA product (~ 2 x 10^8 cells, 1-3 mL of DLA product) compared to peripheral blood (15 mL) prior to ICI treatment. There was a trend towards further increase in the CTC detection frequency in DLA product (18 mL) when an immunodensity-based cell enrichment (RosetteSep™, Stemcell Technologies) procedure (p=0.09) was used. After treatment, no differences were observed in the percentage of patients with CTCs detected in peripheral blood or DLA product. The cell enrichment by RosetteSep™ again increased the yield of CTCs in DLA product (p=0.007, Friedman's 2-way ANOVA by rank).

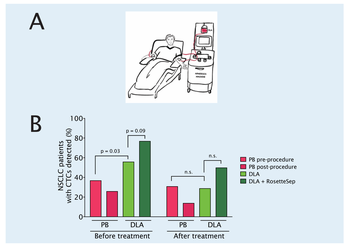

Figure 3. Establishing new workflows for improving the CTC yield in NSCLC patients using Diagnostic Leukapheresis (DLA). (A) Schematic drawing of DLA. **(B)** The percentage of patients with CTCs detected in peripheral blood (PB) prior to and after DLA procedure and in DLA product with and without RosetteSep™ enrichment. The percentage of NSCLC patients with CTCs was assessed in peripheral blood and DLA products before and after treatment with standard-of-care chemotherapy, tyrosine kinase inhibitors or ICI using CELLSEARCH®. Statistical analyses were performed using Fischer's exact test (n.s., not significant).

Improved patient selection for immune checkpoint inhibition (ICI) treatments

- The CANCER-ID partners are currently jointly analyzing samples from advanced NSCLC and metastatic breast cancer patients who are participating in clinical studies at clinical partner sites, or are under ICI treatment at clinics (**Table 1** and **Table 2**). The samples will be collected at baseline and 1-2 and 4-6 months after treatment initiation (**Fig. 4**).
- The aim of this study is to investigate whether CTC counts or mutational analysis of ctDNA using next-generation sequencing (NGS) panels combined with droplet digital PCR (ddPCR) can be used for the selection of patients who may benefit from PD-1/PD-L1 inhibition and identify early signs of efficacy or relapse.
- This approach is supported by the results obtained from NSCLC patients treated with checkpoint inhibitors, a study performed at UMCG (**Table 1**).
- In this study, CTC presence at baseline and CTC change after therapy was used as a stratification tool, and the percentage of early responders (partial and complete response according to RECIST 1.1) and durable responders (stable disease, partial response and complete response according to RECIST 1.1 without progression in 6 months) to ICIs was determined.
- Early response rates were not significantly different (T0: odds ratio, OR=0.67, p=0.56; ΔCTC OR=0.13, p=0.08), whereas, the durable response rate was significantly decreased in patients with CTCs (T0: OR=0.28, p=0.02; ΔCTC OR=0.04, p<0.01) (**Fig. 5**).
- Preliminary data show that a decline in the ctDNA mutation variant allele frequency (VAF) predicts progression-free survival (PFS) and overall survival (OS) (**Fig. 6**).

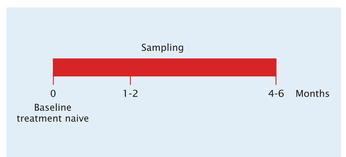

Figure 4. Sample collection workflow for the project aiming for improved stratification of patients for ICI treatments.

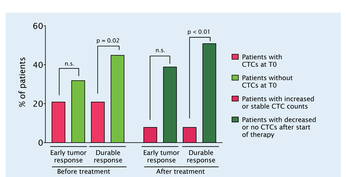

Figure 5. The percentage of early and durable responders in NSCLC patients treated with ICIs, stratified for CTC presence at baseline and CTC change after therapy. The bars represent the percentage of patients (n = 104 before treatment, n = 63 after treatment) with or without circulating tumor cells (CTC) at baseline or with increased or stable CTC counts (ΔCTC) at four to six weeks of therapy compared to baseline. CELLSEARCH® analysis was performed using 7.5 mL of blood and a corresponding volume of DLA product based on the white blood cell count. Statistical analyses were performed using Fischer's exact test (n.s., not significant).

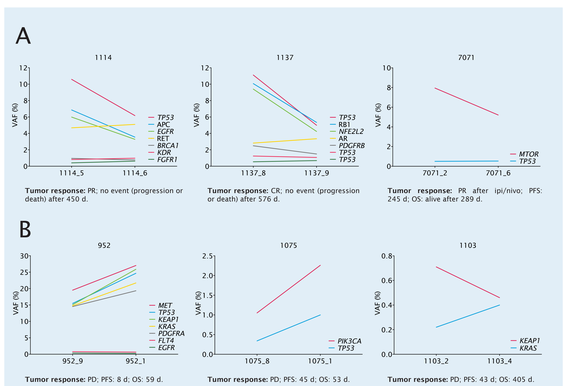

Figure 6. Decline in ctDNA mutation variant allele frequency (VAF) predicts progression-free survival (PFS) and overall survival (OS). (A) Preliminary analysis indicates that stabilization or decline in VAF of NGS-identified mutations in ICI-treated patients is predictive of partial response or stable disease under treatment. **(B)** Rising VAFs are predictive of progressive disease. The AVENIO Expanded ctDNA NGS Analysis Kit (Roche), a pan-cancer NGS panel with 77 genes, was used to identify mutations in advanced NSCLC patients at baseline and approximately 4 weeks into treatment. From 7.5 mL of blood an average of 10 ng of circulating-free DNA was extracted and mutations from multiple tumor clones were detected. CR, complete response; PR, partial response; PD, progressive disease; ipi, ipilimumab; nivo; nivolumab.

Table 1. Characteristics of the ICI study in NSCLC patients.

Paired analysis of 180 NSCLC patients (UMCG Groningen, The Netherlands)
112 patients documented – median age 65 yrs (range 29-83 yrs)
Biopsy available from 100 patients
Gender: Male, Female (N=112) · Histology: Adenocarcinoma, Carcinosarcoma, Squamous cell carcinoma (N=111) · PD-L1 expression: 0 %, 1-50 %, >50 % (N=65)
Tumor profiling: No mutation, KRAS, FGFR1, ALK, BRAF, Other (N=100) · Therapy: Nivolumab, Pembrolizumab, Atezolizumab, Ipilimumab/ nivolumab (N=111) · Response: CR, PR, SD, PD, NE (N=113)
Outcome in tumor response measured with RECIST v1.1, progression-free survival (PFS) and overall survival (OS) is well-documented
Corresponding data on CTCs and tumor-derived extracellular vesicles (tdEV) is available

Table 2. Characteristics of ALICE and ICON phase IIb breast cancer studies.

ALICE, phase IIb, trial NCT03164993		ICON, phase IIb, trial NCT03409198	
Randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of atezolizumab when combined with immunogenic chemotherapy* in subjects with metastatic TNBC		Randomized study evaluating the safety and efficacy of combining nivolumab and ipilimumab with immunogenic chemotherapy* in subjects with metastatic luminal B breast cancer	
Total of 75 patients randomized 2:3		Total of 75 patients randomized 2:3 in favour of arm B, stratified according to tumor PD-L1 status	
Arm A (n=30): Chemo + placebo	Arm B (n=45): Chemo + atezolizumab	Arm A: Chemo only	Arm B: Chemo + ipilimumab + nivolumab
Primary objectives: assessment of clinical response (PFS) and toxicity of combined treatment			
Secondary objectives (among others): assessment of clinical response (ORR, DR, DRR > 6 months, OS) and identification of biomarkers for clinical response, toxicity and immune response			

* Chemotherapy: pegylated liposomal doxorubicin + cyclophosphamide
DR, duration of response; DRR, durable tumor response rate; ORR, overall tumor response rate; OS, overall survival

The European Liquid Biopsy Society

- IMI's CANCER-ID project ends in December 2019. The requirement for continued data and sample storage, further updating of best practice documents and standard operating procedures (SOPs) and scientific support of liquid biopsy proficiency testing have led to plans for sustained activity in the field by academic and industrial partners.
- The University Medical Center Hamburg-Eppendorf (UKE, Hamburg) is currently establishing the "European Liquid Biopsy Society" (ELBS) (**Fig. 7**) with the following goals:
  - Foster the introduction of liquid biopsy into clinical practice.
  - Encourage interactions between academia and industry as well as other related initiatives (e.g. The US-based Blood Profiling Atlas in Cancer, BloodPAC; FNIH).
  - Provide a partner for regulatory agencies, healthcare providers and patient advocacy groups.
  - Support the implementation of liquid biopsy tests into clinical trials.
  - Develop guidelines and provide training in liquid biopsy for medical scientists.
  - Disseminate knowledge about liquid biopsies to the medical community through regular symposia, publications and press releases.
- International stakeholders worldwide are cordially invited to join the ELBS and support the advancement of liquid biopsy in cancer research and therapy. A kick-off meeting is being planned for May 3rd 2019 at UKE in Hamburg, Germany. For additional information please contact Prof. Klaus Pantel (pantel@uke.de).

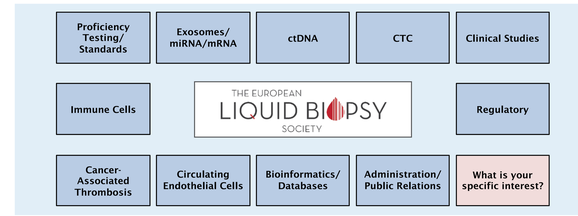

Figure 7. Scope of the European Liquid Biopsy Society (ELBS).

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2. Brudzewsky *et al.* Multicenter evaluation of circulating tumor DNA assays. Session Category: Clinical Research; Session Title: Current Developments in Non-invasive Biomarkers for Assessment of Cancer 1; Session Date and Time: Sunday Mar 31, 2019 1:00 PM - 5:00 PM; Location: Exhibit Hall B, Poster Section 18; Permanent Abstract Number: 438.

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